Innervation of the Human Periodontal Membrane and Gingiva

William D. Kirstine D.D.S.

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Orthodontics.

Horace H. Rackham School of Graduate Studies
University of Michigan
Ann Arbor, Michigan
1957

ACKNOWLEDGEMENTS

I would like to thank Professor Robert E. Moyers, head of the Department of Orthodontics, School of Dentistry, University of Michigan, for his encouragement during the course of this study.

To Assistant Professor James K. Avery, I wish to express my deep gratitude for his inspiration, guidance and assistance in the preparations of this thesis and also for placing the facilities of the Department of Oral Anatomy at my disposal.

I wish also to thank Dr. Robert Rapp who has offered much valuable assistance throughout the entire study.

Further thanks must be extended to Miss Elizabeth W. Haley for her technical assistance.

TABLE OF CONTENTS

INTRODUCTION

The innervation of the periodontal membrane and gingiva has been the object of considerable study during the past fifty years. However, the findings and conclusions of various investigators differ and are often contradictory. Most of the past investigations have been carried out on a number of different animals and many of the findings have been presented in the form of drawings and verbal descriptions. It is difficult to compare the various observations and moreover to relate them to the findings in man. As a result, the knowledge concerning innervation of the human periodontal membrane and gingiva is inadequate and controversial.

The purpose of this investigation is to gain additional knowledge of the innervation of the human periodontal membrane and gingiva and thus to attempt to clarify the conflicting literature on this subject.

The present investigation consists of two parts. The first part is a study of the morphology and distribution of the neural structures in the human periodontal membrane and gingiva, by the method of silver impregnation. The second is a study of the presence and the histochemical localization of the enzyme acetylcholinesterase in the gingiva. In this manner the comparative location of the silver stained nerve fibers with acetylcholinesterase could be made. It was also believed that the presence of a cholinergic mechanism in the

human gingiva might allow inferences to be drawn regarding the mechanism of function of the neural elements in that tissue.

REVIEW OF THE LITERATURE

(a) Innervation of the Periodontal Membrane and Gingiva

Investigation of the literature concerning innervation of the periodontal membrane and gingiva has brought to light a considerable volume of material. In order to present a brief account of the various observations of the earlier investigators, Table I has been constructed. However, certain of these studies are more pertinent to the present investigation than others and will thus be discussed in a more detailed manner.

Periodontal Membrane

In 1913 Dependorf[10] found the periodontal membrane of extracted human teeth to contain nerve bundles in association with blood vessels running centrally in the periodontal membrane parallel to the long axis of the tooth. He noted that these fibers arise from the apical area of the teeth and are joined in their course by fibers from the alveolar bone. The nerve bundles found in the periodontal membrane form both coarse and fine networks which terminate as fine pointed processes in the cementoblastic layer of the teeth, the medullated spaces of the bone and the gingival papillae.

Kadnoff* , in 1929, studied the innervation of the

* Not read in the original; refer to reference number 20.

periodontal membrane of human teeth. He found that the nerve fibers which arise at the apical area of the tooth pass vertically towards the gingiva being reinforced at intervals by nerve bundles entering the periodontal membrane through foramina in the alveolar bone. These fibers were noted to terminate as a fine network in the membrane proper without entering the cementum. No encapsulated nerve endings were demonstrated.

In 1936 Van der Sprenkel[29] described three types of nerve endings in the periodontal membrane of the mouse: (1) small end rings, surrounded by a periterminal reticulum, which were situated near the alveolar bone in conjunction with collagenous fibers passing into the cementum; (2) terminal reticular endings in close association with connective tissue cells; and (3) reticula with radial fibers that penetrate into the cementum and in some cases into the dentinal tubules.

The work of Bradlaw[6] in 1936 on man, monkey, dog, cat and guinea pig demonstrated that the nerves of the human periodontal membrane pass upwards in association with blood vessels and at various levels give off branches to the surrounding alveolar bone. The main nerve bundles extended to the alveolar crest and there anastomosed with nerves in the gingiva and nerves in the periodontal membranes of adjacent teeth. Bradlaw also noted terminal coils in the periodontal membrane and loops formed by the neurofibrils turning back from the cementum.

Lewinsky and Stewart[19] in 1936 noted that the nerve fibers in the periodontal membrane of man end in fine arborizations, many of which have a terminal knob like body. No fibers could be traced into the cementum of the teeth.

In 1937 Lewinsky and Stewart[20] studied the periodontal membrane of the cat. They noted that the nerve fibers in the periodontal membrane are of two types. The first were thick fibers confined to the peripheral part of the membrane which had specialized end organs at their termination. These end organs were spindle-like in shape and were formed by the nerve fiber becoming twisted like a spiral spring. At intervals on the convolutions they observed rounded thickenings. The second type were fine nerve fibers which pass to the deeper parts of the periodontal membrane and break up into fine arborizations without terminal organs.

Bernick[2,3,4,5] in 1952, 1955, 1956 and 1957 demonstrated in man, the monkey and the rat that the innervation of the periodontal membrane arises from the apical area of the tooth and the interalveolar nerves. He noted that the periodontal nerves terminate as "free nerve endings" or spindle shaped endings among the cells of the periodontal membrane, the cementoblastic layer and in the cementum itself.

In 1956 Gach[11] investigated the innervation of the periodontal membrane of the Syrian hamster. Numerous terminal nerve endings of both the organized and unorganized types were noted but no encapsulated endings were found.

The Gingiva

Jurjewa* in 1913 described two types of nerve endings in the gingiva of the cat, rabbit, horse, cow and mouse: (1) encapsulated endings situated in the subepithelial connective tissue and similar in morphology to the tactile corpuscle found in other mucous membranes. (2) non-encapsulated endings which consisted of a network of intermingling thick and thin nerve fibers localized in the connective tissue papillae between the rete pegs of the epithelium.

In 1928 Kadnoff* found both non-encapsulated and encapsulated types as appearing to terminate in either a close or loose coil. The close coil endings consisted of both thick and thin nerve fibers arranged in tight balls situated in the connective tissue papillae. Terminal nerve fibers emerged from these balls and entered the epithelium. The loose coils, on the other hand, arose from the subpapillary layer and passed into the papillae as loops which filled the domes of the papillae. He found two types of encapsulated endings deep in the subepithelial tissue: (1) Pacinian corpuscles; and (2) end bulbs of Krause.

In 1938 Stewart and Lewinsky[28] made a comparative study of the innervation of the gingiva of the cat, ferret, mouse, rabbit, mole and human. They concluded that a deep or

* Not read in the original; refer to reference number 2.

superficial nerve plexus was present in all animals studied. Both loose and close intrapapillary nerve coils were noted in man.

Bernick[4,5] in 1956 and 1957 studied the gingiva of the rat, monkey and man but was unable to locate any type of specialized or encapsulated nerve endings.

In 1950, Gairns and Aitchison[12] found the human gingiva to be highly innervated, but noted that the frequency of occurrence of endings varies greatly between different areas of the gingiva. Many types of specialized and encapsulated nerve endings were seen in the dermal papillae including typical Meissner corpuscles. They demonstrated a more frequent penetration of the epithelium by nerve fibers than heretofore suggested. Krause corpuscles were frequently seen in the dermis below the papillae and occasionally in the dermal papillae themselves. Free nerve endings located immediately below the epidermis frequently terminated in the deeper layers of the epithelium.

TABLE I

INNERVATION OF THE PERIODONTAL MEMBRANE AND GINGIVA

Author	Ref. No.	Date	Specimen	Observations
Dependorf	10	1915	Human	1. Nerve bundles in association with blood vessels running centrally in the periodontal membrane. 2. Free nerve endings in the periodontal membrane and the cementoblastic layer. 3. Periodontal nerves terminating within the medullated spaces of the bone and the gingival papillae in the form of simple processes. 4. Anastomosis between the nerves of the periodontium and those of the bone and gingiva.
Stewart, D.	27	1927	Human Cat	1. The nerves of the periodontal membrane are the organs of tactile sensation for the teeth.
Mowry, D. P.	24	1930	Human gingiva	1. Nerve bundles usually associated with blood vessels in the connective tissue beneath the oral epithelium. 2. Non-medullated nerve fibrils penetrating between the cells of the basement layer of the epithelium and extending irregularly towards the surface.
Bradlaw, R.	6	1936	Human Monkey Dog Cat Guinea pig	1. Main nerve bundles accompany blood vessels in a gingival direction in the periodontal membrane. 2. Periodontal nerves terminate as loops and coils supplying the alveolar bone and cementum. 3. Periodontal nerves extend to the alveolar crest and there anastomose with nerves in the gingivae and periodontal membranes of adjacent teeth.

TABLE I--Continued

Author	Ref. No.	Date	Specimen	Observations
Brashear, A. D.	8	1936	Human Cat	1. Bundles of nerve fibers enter the periodontal membrane in association with blood vessels, some entering the membrane through the alveolar bone. 2. Wide range of fiber sizes in the periodontal membrane. 3. Sensations other than touch may be elicited from the periodontal membrane.
Lewinsky, W. Stewart, D.	19	1936	Human	1. Nerve fibers of the periodontal membrane come from the apical region of the tooth and run towards the gingiva in longitudinal bundles in company with blood vessels. 2. Nerve bundles are reinforced in their course by fasciculi which enter the membrane through foramina in the alveolar process. 3. The nerve fibers of the periodontal membrane end in fine arborizations, many of which have a terminal knob-like body. 4. No fibers could be traced into the cementum of the teeth.
Van der Sprenkel, B.	29	1936	Human Mouse	1. Periodontal nerves lie in close relation to the alveolar bone as they pass towards the gingival margin. 2. Periodontal nerves originate from the apical area of the teeth and from foramina in the alveolar bone at points between the apex and alveolar crest.

TABLE I--Continued

Author	Ref. No.	Date	Specimen	Observations
Van der Sprenkel (Cont.)				3. Three types of nerve endings are found in the periodontal membrane: (1) terminal reticular endings in close relation to connective tissue cells; (2) small end rings close to the alveolar bone; and (3) centrally located axons that sometimes penetrate and terminate in dentinal tubules.
Lewinsky, W. Stewart, D.	20	1937	Cat	1. Nerve fibers arising from foramina in the alveolar bone appear to form a greater proportion of the innervation of the periodontal membrane than has hitherto been believed. 2. Nerve fibers in the periodontal membrane are of two types: (1) thick fibers confined to the peripheral part of the membrane which have specialized spindle shaped end organs at their termination; (2) fine nerve fibers which pass to the deeper part of the periodontal membrane and break up into fine arborizations without terminal organs. 3. No fibers could be traced into the cementum.
Lewinsky, W. Stewart, D.	21	1938	Human	1. Found both large and small close coils located near the apices of the gingival papillae. 2. The fibers forming the large close coils did not branch but simply twisted and coiled upon each other. 3. A thick intra-epithelial fibre originated from the small close coils.

TABLE I--Continued

Author	Ref. No.	Date	Specimen	Observations
Lewinsky Stewart (Cont.)				4. Loose coils or networks, occupying the whole of the papilla. Fibers forming these coils branch, and from the coils arose fine intra-epithelial fibers. 5. Fine epithelial fibers arose from a subepithelial nerve which was reinforced at intervals by nerve loops.
Bradlaw, R.	7	1939	Human Sheep Cat Monkey	1. Presence of a perivascular neural plexus in the periodontal membrane. 2. In man, intra-epithelial fibers which pass from intra-papillary neural coils to terminate near the surface of the epithelium in knob-like endings. 3. Thickening and varicose changes of the pulpal and periodontal nerves in periodontal disease.
Stewart, D. Lewinsky, W.	28	1939	Human Cat Ferret Mouse Mole Rabbit	1. The nerve supply of the gingiva is partially derived from the periodontal membrane. 2. Specialized nerve endings occur in the connective tissue of the gingiva. 3. Coiled nerve endings are found both in the intrapapillary zone of the gingiva and in the deeper connective tissue.
Gairns, F. W. Aitchison, J.	12	1950	Human gingiva	1. The gingiva is extremely well innervated. 2. Many typical Krause corpuscles are found in all levels of the dermis below the papillae and occasionally in the papillae themselves.

TABLE I--Continued

Author	Ref. No.	Date	Specimen	Observations
Gairns Aitchison (Cont.)				3. Immediately below the epidermis there are often to be found many myelinated nerves which, breaking into smaller twigs by repeated dichotomous branchings, end in little knob-like thickenings or end loops. 4. There exists a very profuse sympathetic ground plexus in the dermis. 5. Many types of specialized nerve endings are found in the dermal papillae including Meissner corpuscles. 6. In the gingiva, there are many nerve endings which send "ultra terminal" fibers to the outermost layers of epithelial cells of the epidermis. 7. No Pacinian corpuscles are found in the human gingiva.
Mohiuddin, A.	23	1950	Cat	1. Degenerative changes can be demonstrated in the periodontal nerves of deciduous teeth in the process of shedding.
Bernick, S.	2	1952	Monkey	1. The nerve supply of the periodontal membrane arises from the dental and interalveolar branches of the alveolar nerves. 2. The periodontal nerves terminate as "free nerve endings" among the cells of the periodontal membrane, the cementoblastic layer, and the cementum proper. 3. No specialized nerve endings such as Pacinian or Meissner's corpuscles were seen either in the periodontal membrane or gingiva.

TABLE I--Continued

Author	Ref. No.	Date	Specimen	Observations
Bernick, S.	3	1955	Rat Monkey	1. Pretreatment of tissues with a proteolytic enzyme, such as pepsin or papain, removes the collagenous elements, and permits a clearer and more accurate identification of nerves.
Gairns, F. W.	13	1955	Human	1. The sensory nerve endings of the adult human hard and soft palate and the uvula consist of free nerve endings within the epithelium, numerous organized endings in the dermal papillae and a few organized endings in the subpapillary region of the dermis.
Held, A. J. Band, C. A.	14	1955	Human	1. In the human gingiva, no nerve fibers were seen penetrating into the epithelium and no encapsulated or even coiled nerve endings were seen.
Bernick, S.	4	1956	Rat	1. The nerves of the periodontal membrane terminate as "free nerve endings." 2. Gingival innervation is derived from nerves of the periodontal membrane and from fibers originating from the labial or palatal nerves. 3. No specialized nerve endings such as Krause's or Meissner's corpuscles were seen in either the periodontal membrane or gingiva.
Gach, L.	11	1956	Syrian hamster	1. Numerous terminal nerve endings of both the organized and unorganized types were noted in the periodontal membrane. 2. In the gingiva, fine arborization extended into the basal and granular layers of the oral epithelium and terminated in fine or blunt endings.

TABLE I--Continued

Author	Ref. No.	Date	Specimen	Observations
Gach (Cont.)				3. No encapsulated nerve endings were seen in either the periodontal membrane or the gingiva.
Bernick, S.	5	1957	Human Monkey	1. Two types of nerve endings are found in the periodontal membrane: (a) "free nerve endings" among the stroma cells, cementoblasts and cementum; (b) medullated fibers may lose their myelin sheath and the naked fibrils terminate as an elongated spindle-like structure. 2. No specialized nerve endings were seen in the gingiva. 3. The attached gingiva contains both papillary and intra-epithelial nerve endings. 4. Intra-papillary and intra-epithelial nerve endings are scarce in the epithelial attachment and marginal gingiva.

(b) The Role of the Cholinergic Mechanism in Neural Function

Investigation of the literature regarding the role of the cholinergic mechanism in the transmission of nerve impulses reveals that our knowledge on this subject at the present time is limited. In the present literature, there are no recorded studies on the presence or absence of this mechanism in the human gingiva or periodontal membrane.

Since the discovery and identification of acetylcholine, problems in the function and distribution of this material have been intensely investigated. However, the part played by acetylcholine in the tramsmission of nerve impulses has not yet been completely elucidated. It is contended by some that acetylcholine is essential for the propagation of nerve impulses, while others hold that acetylcholine acts only at certain cholinergic endings.

Koelle[16] in 1951 states that a nerve impulse in a cholinergic neuron resulting from the progressive depolarization along the membrane of the nerve probably results in the liberation of acetylcholine along its entire length. At preganglionic terminations or motor end plates, the larger quantities of acetylcholine liberated by the numerous ramifications could diffuse to the specialized region of the post-synaptic element to produce a localized depolarization, which in turn results in the formation of a nerve impulse. The acetylcholine is then hydrolyzed and de-activated by acetylcholinesterase, thus stopping

nerve impulse transmission.

Actually, there are two esterases capable of hydrolyzing acetylcholine. One of these is a non-specific esterase found in various animal tissues such as blood serum, pancreas, and liver. The other is the specific cholinesterase or acetylcholinesterase found in nerve fibers which hydrolyzes only acetylcholine.[18]

Koelle and Friedenwald[15] in 1949 developed a histochemical technique for localizing cholinesterase activity. They showed that non-specific cholinesterase and specific cholinesterase can be readily differentiated in tissues by their relative hydrolytic activities on certain esters under carefully controlled conditions.

In 1955, Malmgren and Sylven[22] investigated and analyzed the chemical mechanism involved in Koelle's technique.

Churchill, Schuknecht and Doran[9] believe that the presence of acetylcholinesterase in the nervous system is strongly presumptive evidence that the cholinergic mechanism operates where the enzyme is found. Acetylcholinesterase concentrations in any given region of the nervous system have, in general, been found to parallel concentrations of acetylcholine. In the past, acetylcholinesterase was thought to be absent from sensory or afferent neurons. However, the number of sites now demonstrated to contain acetylcholinesterase have been increased to the point where this enzyme might be considered almost universally distributed in the nervous system.

It is known, however, that the quantities of acetylcholinesterase in different neural structures differ widely. Koelle[17] in 1955 demonstrated the variable concentrations of acetylcholinesterase in adrenergic, sensory and cholinergic neurons of the cat, rabbit and Rhesus monkey. On the basis of his findings, Koelle suggests that the terms cholinergic and adrenergic may refer to the predominant but not necessarily the exclusive transmitting agents of the respective nerve fibers.

MATERIALS

In order to carry out an investigation of this type, it is necessary to have a readily available source of fresh material. All the fresh tissues used in this study were obtained from the departments of Oral Surgery and Periodontia of the School of Dentistry of the University of Michigan.

The maxilla of a two and one-half year old child was obtained from the department of Oral Anatomy.

Freshly extracted human teeth which had portions of the alveolus attached and human gingival tissue removed in gingivectomy procedures were obtained from the departments of Oral Surgery and Periodontia respectively. As soon as a fresh sample of tissue was procured it was immediately placed in an appropriate fixative.

It is realized that in studying the periodontal membrane and gingiva of extracted teeth with fragments of adherent bone, that during extraction damage may have been done to such fine structures as nerve fibers. However, study of the tissue revealed no evidence of such an occurrence and it has been possible to trace fine nerve fibers for a considerable distance without discovering any sign of rupture.

METHODS

(a) Demonstration of Neural Elements by the Method of Silver Impregnation

Fresh periodontal membrane and gingival tissue were fixed in a 10% formalin solution for approximately three days. After fixation the tissue were decalcified if necessary in a solution consisting of equal parts of 20% sodium citrate and 50% formic acid. After being dehydrated, the tissues were cleared and embedded in paraffin. Sections were then cut ranging in thickness from fifteen to fifty micra. Thin sections show greater cellular detail while thicker sections give greater continuity to the nerve fibers.

After mounting, the tissues were stained using Powers[22] modification of Romanes[23] silver nitrate method. This technique is illustrated in detail in Table II.

Before staining, it was found essential to mordant the sections in a .5% cupric nitrate solution overnight in a 37° C. oven. This resulted in diminished background staining and intensified the staining of the neural elements.

In tissues stained by this method, the nerve fibers are stained black in contrast to the much lighter staining of the surrounding non-nervous structures. The use of this technique with serial sections allows the course of the nerve fibers to be clearly followed through the various tissues.

However, it must be stressed that simply because a

structure stains with silver is no proof that the structure is a nerve. Silver may stain non-nervous structures rather selectively. Unless the structure has the morphology of a nerve fiber the possibility of non-specific staining or of artifact must be considered.

TABLE II

POWERS MODIFICATION OF ROMANES SILVER NITRATE TECHNIQUE

Fix	10% formalin for 1-3 days.
Rinse	Wash in running tap water.
Decalcify	Equal parts of 20% sodium citrate and 50% formic acid.
Rinse	Wash in running tap water.
Dehydrate	Dehydrate slowly using 50%, 70%, 80%, 95%, 100% ethyl alcohol.
Clear	Dioxane -- 6 hours.
Embed	Infiltrate with paraffin at 56-58°C.
Section Mount	15-50 micra. Place tissues on slide using albumen fixative.
Hydrate	Remove paraffin with xylol and hydrate through graded ethyl alcohols to distilled water.
Mordant	Place in 0.5% aqueous cupric nitrate solution for 6-16 hours in 37°C oven.
Rinse	Rinse through 5 or 6 changes of distilled water.
Stain	10-12 hours in the following solution in 56°C oven: 0.1% $AgNO_3$ - 2.9 cc 0.1% NaCl - 1.0 cc 1.0% NH_3 - 16 drops Distilled H_2O - 50 cc
Rinse	Distilled H_2O for 30 seconds.
Develop	For 5 minutes in the following solution: Hydroquinone - 1.0 g Sodium sulfite-5.0 g Distilled H_2O -100.0 ml
Rinse	Through two changes of tap water and distilled H_2O.
Tone	For 10 minutes in a 0.2% solution of gold chloride.

TABLE II--Continued

Rinse	In distilled water for 2 minutes.
Immerse	Place in 0.5% or 1% oxalic acid until the nerves are clearly defined (not more than 3 minutes). This is a critical stage. All tissues apparently react differently. Pilot slides should be run through and nerve differentiation checked under a microscope.
Rinse	Distilled water for 1 minute (stops action of oxalic acid).
Fix	In 5% sodium thiosulfate for 5 minutes.
Rinse	In tap water and distilled H_2O.
Staining	Eosin or other suitable counterstains, may be used.
Dehydrate	Using changes of 50%, 70%, 80%, 95%, 100% ethyl alcohol.
Clear	In xylene or toluene.
Mount	Clarite.

(b) Histochemical Technique for Demonstration of the Presence of Acetylcholinesterase

Sites of cholinesterase activity were localized using the Avery, Rapp[1] modification of the histochemical technique first presented by Koelle[15].

The principle of this technique, in brief, consists of incubating fresh tissues in a solution containing cupric ions. At points of cholinesterase activity, the substrate is hydrolyzed, and the substance liberated forms copper thiocholine. This precipitate is subsequently converted to copper sulfide when the specimen is treated with ammonium sulfide. Copper sulfide, a rather stable, brownish black precipitate is seen wherever the enzyme cholinesterase has been acting.

Separation of the specific and non-specific cholinesterases is accomplished by the use of selective inhibitors and substrates.

Methodology of the Avery, Rapp technique (Table VI) is as follows.

The tissues to be studied are collected and immediately placed in a normal saline solution. Soft tissues may be treated either in bulk form or by sectioning in a freezing microtome at fifty micra. Calcified tissues such as teeth and bone are sectioned on a water cooled disk and further reduced in size by grinding on a water cooled abrasive wheel. The frozen sectioned tissues are placed on albuminized slides and allowed to dry for approximately one minute.

The tissues are then transferred as quickly as possible to the appropriate storage solutions, B or C. (Table IV). Tissues to be treated with an inhibitor are placed in this solution while the other tissues are retained in the storage solution. Di-isopropyl fluorophosphate inhibition is carried on for thirty minutes at 35° C.

Following inhibition, the tissues are rinsed in the appropriate storage solutions and then transferred to the incubation solutions (Table III). Incubation times may range from five minutes to twenty-four hours at 38° C (Koelle) or 37° C.

After incubation the tissues are carried through three rinsing solutions (Table V), and then treated with ammonium sulfide solution for twenty seconds (Koelle) or until black staining becomes apparent. The copper thiocholine precipitate which appeared at the sites of acetylcholinesterase activity after incubation is thus converted to a brownish black precipitate of copper sulfide. After being developed, the tissues are rinsed in distilled water for one second and then fixed in copper sulfide saturated 10% neutral formalin for thirty minutes.

Next the tissues are dehydrated in an ascending series of alcohol solutions containing traces of copper thiocholine. The tissues are cleared in xylene or methyl salicylate containing traces of copper sulfide.

Soft tissues are then paraffin embedded, sectioned at

thirty to fifty micra, hydrated to 70% alcohol, counter-stained with eosin, dehydrated and mounted in Canada balsam. Teeth are mounted directly in the Canada balsam or may be decalcified, embedded with paraffin, sectioned and mounted.

TABLE III

INCUBATION SOLUTIONS

Incubation Solution	Na_2SO_4 ml	Cu-Gl ml	Mal. ml.	$MgCl_2$ ml	CuThCh ml	AThCh ml	BuThCh ml	H_2O ml
B	9.0	0.6	1.5	0.6	Trace	1.2	-	2.1
C	10.5	0.6	1.5	0.6	Trace	-	1.2	0.6
D	6.0	0.4	1.0	0.4	Trace	-	0.8	1.4
E	6.0	0.4	1.0	0.4	Trace	-	-	1.4

TABLE IV

STORAGE SOLUTIONS

Storage Solution	Distilled H_2O	40% Na_2SO_4
B	6.0 ml	9.0 ml
C	4.5 ml	10.5 ml

TABLE V

RINSING SOLUTIONS

Solution	Na_2SO_4 (40%)	H_2O	CuThCh	Time
1	10.0 ml	10.0 ml	Saturate	5 min.
2	5.0 ml	15.0 ml	Saturate	1 min.
3	-	20.0 ml	Saturate	1 min.

TABLE VI

USE OF INHIBITOR, STORAGE SOLUTIONS, AND INCUBATION SOLUTIONS REQUIRED TO LOCALIZE THE ENZYMES

Storage Solution	Inhibitor	Incubation Solution	Cholinesterases Stained		Cholinesterases Inhibited	
			Ach E	N-S	Ach E	N-S
B	-	B	+	+	-	-
B	Diisopropyl fluorophosphate	B	+	-	-	+
C	-	C	-	+	+	-
B	D. F. P.	D	-	-	+	+
B	-	E	-	-	+	+

OBSERVATIONS

(a) The Periodontal Membrane

The nerves of the periodontal membrane arose from two sources (Figure 2). The main nerve fibers originated in the apical region of the alveolus and ran towards the gingiva in longitudinal bundles (Figure 1). These fibers were branches of the dental nerve given off before the nerve entered the root apex. The second group of fiber bundles entered the periodontal membrane through foramina in the alveolar bone at varying levels between the apical region of the alveolus and the alveolar crest (Figure 2). Some of these accessory fibers from the alveolar bone turned towards the apical region of the tooth while others turned towards the gingiva (Figure 2).

From the periodontal fibers in the apical region of the tooth, small nerve twigs arose which supplied the periapical tissue. The terminal branches of these twigs ended either in close relation to the stroma cells of the periodontal membrane or the cementoblasts (Figure 3). The perforating fibers from the alveolar bone after entering the periodontal membrane gave off small twigs which supplied the periosteal surfaces of the membrane adjacent to the alveolar bone. The remaining alveolar fibers communicated with the fibers from the apical region and with each other forming a neural plexus throughout the membrane (Figure 8).

The main nerve bundles were located centrally in the periodontal membrane, while a lesser number of bundles were found lying in close relation to the root and the alveolar bone (Figures 1 and 8). The larger nerve bundles were usually associated with blood vessels, while many of the smaller bundles and single fibers showed no such relationship (Figures 1 and 9).

The nerve bundles passed gingivally through the periodontal membrane to the level of the alveolar crest. In this area, anastomoses occurred between the nerve fibers of the periodontal membranes of adjacent teeth and between the nerves of the periodontal membrane and subpapillary connective tissue of the gingiva. A few of the periodontal fibers passed directly to the free gingiva and terminated in the papillary and reticular connective tissue (Figure 17).

At intervals throughout the course of the nerve bundles in the periodontal membrane, fibers were seen to terminate among the fibroblasts of the periodontal membrane, in interstitial spaces throughout the membrane, and in close relation to the alveolar bone and cementum (Figures 6, 7 and 9). A variety of free nerve endings and specialized nerve terminations were observed. These nerve endings showed a great deal of variation in structure and location (Figures 4,5,6, and 7).

Free nerve endings were seen to terminate throughout the periodontal membrane from near the cemental surface of the root to the alveolar bone. These fibers terminated with and

without end swellings, and with and without arborizations. Some of the free nerve endings formed a little hook just prior to their termination (Figures 8 and 9).

Highly organized neural structures were seen in some of the interstitial spaces of the periodontal membrane (Figure 5). These structures were large and ovoid in shape. They consisted of medullated and non-medullated nerve fibers which were twisted and interwoven among themselves, forming closely related loops and convolutions. The connective tissue immediately around the nerve groupings showed a somewhat different organization than elsewhere in the periodontal membrane. It appeared less fibrous and the fibers appeared to be oriented around the periphery of the neural structure (Figure 5).

In the central portion of the periodontal membrane near the level of the alveolar crest, specialized nerve endings were seen in close association with blood vessels (Figure 4). The nerve endings consisted of a small nerve loop located in a connective tissue structure which had a definite capsular appearance. Although these structures could only be viewed in one plane, it was theorized that the ending is spherical in shape with a nerve loop enclosed within the periphery of an altered connective tissue area.

In many areas of the periodontal membrane, medullated nerve fibers were seen to lose their myelin sheath. The naked nerve fibrils then terminated as slight knob like swellings, fine arborizations or spindle shaped structures (Figures 8 and 9).

In other instances, medullated fibers were seen to terminate abruptly among the fibroblasts of the periodontal membrane as blunt, club shaped endings (Figures 6 and 7).

Some nerve bundles divided repeatedly into fine fibers, and then terminated in convolutions and loops of varying size. In these areas, no alteration in structure of the adjacent connective tissue was noted (Figure 9). Other dividing fibers were seen to radiate at an acute angle from the main fiber bundle towards the periphery of the periodontal membrane. Here they terminated as fine ramifications (Figure 7).

In some areas single nerve fibers extended from deep in the periodontal membrane towards the surface of the cementum (Figure 3). Many of these fibers terminated as small loops and coils in close relation to the cementoblasts, while others were noted to turn back from the cementum and terminate abruptly as blunt endings (Figures 6 and 7). In no instance, was a nerve fiber observed penetrating the cementoblastic layer or the cementum proper.

(b) The Gingiva

Gingival innervation is derived from two sources. Nerve bundles originating from the labial or buccal and lingual or palatal nerves comprise the main source of supply. These nerves were seen to originate deep in the sub-epithelial connective tissue. From here, the nerve fibers course towards the epithelium. As the fiber bundles pass towards the surface of

the epithelium, branches are given off which terminate in the sub-papillary connective tissue, the sub-epithelial papillae and the epithelium itself (Figures 10,11 and 12).

A secondary source of gingival innervation is the periodontal membrane. A comparatively few nerve fibers from the periodontal membrane, after supplying that structure, pass to the free gingiva. Here they terminate in the sub-epithelial connective tissue and occasionally in the epithelium itself.

As in the periodontal membrane, the larger fiber bundles in the gingiva were often seen in close association with blood vessels (Figure 17), while the smaller ones did not always present this relationship (Figure 10).

The gingiva was extremely well innervated and contained a large variety of nerve terminations (Figure 12). However, the frequency of occurrence of the nerve endings varied greatly between different areas of the gingiva. Intra-papillary and intra-epithelial nerve endings were scarce in the epithelial attachment and free gingiva. On the other hand, the attached gingiva contained many of both types of nerve endings (Figure 12).

For the sake of convenience and clarity, it is probably best to describe the nerve endings observed according to the area of the gingiva in which they occurred.

In the sub-epithelial connective tissue two types of nerve endings were observed. Deep in the submucosa, a large highly organized neural structure was seen (Figure 8).

It was oval in shape, quite ~~large~~ in size and appeared similar in structure to the specialized ~~nerve~~ groupings observed in the interstitial spaces of the periodontal membrane (Figure 5). The main body of the structure appeared to consist solely of myelinated nerve fibers in the form of loops and convolutions. However, fine non-medullated fibers were seen entering one end of the ovoid body.

Immediately beneath the epithelium were seen many myelinated nerve fibers. Often these fibers divided into small twigs by repeated dichotomous branchings, to end in fine arborizations (Figure 10). The finest fibers were occasionally seen to enter the epithelium where they ramified around the cells of the deeper layers (Figure 11).

The sub-epithelial papillae of the gingiva are profusely innervated and are the site where most of the peculiarities in the nature of the terminal nerve endings occur. (Figure 12). In many instances four and five adjacent papillae were seen to contain nerve endings which varied in size and shape and were either encapsulated or non-encapsulated (Figure 12). It is difficult to identify and describe many of the endings in the papillae since they are of such diverse form and size. However, it is quite possible that many of these terminal endings which appear to differ in form and structure are in reality quite similar. Sectioning of similar endings on different planes of reference could be the cause of the apparent differences.

Three types of encapsulated nerve endings were seen in the sub-epithelial papillae. Typical Meissner's corpuscles were frequently seen (Figures 12,13 and 16). These end bulbs were somewhat ovoid structures made up of a central mass of irregular cells penetrated by irregularly curved nerve endings. They possessed a many layered capsule of connective tissue.

Krause end bulbs were also seen in the sub-epithelial papillae (Figure 12). However, they occurred less frequently than endings of the Meissner type. The Krause end bulb was larger and more round than the other endings observed. A single large nerve fiber was seen entering the bulb. Inside the bulb the nerve fiber branched repeatedly and ended in several free enlarged terminations.

The third type of encapsulated ending was classified simply as a closed coil (Figures 14 and 15). The end bulb was much smaller than the Meissner and Krause types, and consisted of closely interwoven nerve fibers arising from a large myelinated nerve fiber in the papilla.

Free nerve endings in the sub-epithelial papillae assumed various forms. The type most frequently seen took the form of an open mesh arising from a nerve fiber in the lamina propria (Figures 17 and 18). Occasionally fibers of the meshwork were seen penetrating the basal cell layer of the epithelium. In no instance were intra-epithelial fibers seen to arise from encapsulated nerve endings in the papillae.

Localization of Acetylcholinesterase in the Gingiva

Gingival tissue incubated in acetylthiocholine without enzyme inhibition contained numerous diffuse deposits of copper sulfide precipitate (Figure 20). These deposits were located throughout the gingiva, but were concentrated most heavily in the lamina propria in areas previously noted to contain nerves and blood vessels. These deposits which were seen in the papillary and reticular connective tissue indicate the presence of the enzymes cholinesterase in these areas.

Incubated gingival tissue treated with an inhibitor (di-isopropyl fluorophosphate) also showed numerous deposits of copper sulfide throughout the gingiva (Figure 21). However, these areas were less numerous and more localized in the papillary c.t. than those previously noted. The finding of these deposits indicated the presence of acetylcholinesterase (specific cholinesterase) in the areas in which they were found. Location of the copper sulfide deposits were seen to correspond closely with areas previously noted to contain nerve fibers and nerve endings.

Gingival tissue incubated without a substrate acted as a control for the above tissues, and was seen to contain no copper sulfide deposits (Figure 19).

DISCUSSION

(a) Periodontal Membrane

The problem of the innervation of the human periodontal membrane and gingiva is a controversial subject, and one on which there is a scarcity of reports in the literature. This is due in part to a lack of suitable material for nerve study. The disagreements noted in the literature that is available are largely the result of technical difficulties leading to varied interpretations of the material studied.

Nearly all studies investigating the innervation of the periodontal membrane agree, as does the present study, that the larger nerves of the periodontal membrane accompany blood vessels along their course through this tissue, while many of the smaller nerves show no such relationship. Moreover, most of the studies agree on the source of the nerves and the paths that they follow in the periodontal membrane. It is in the mode of branching and the final termination of the periodontal nerves where the controversy exists.

In this study, the finding of free nerve endings which terminated throughout the periodontal membrane from near the cemental surface of the root to the alveolar bone, is in agreement with the observations of Dependorf[10] on man, Bernick[2] on the monkey and Van der Sprenkel[29] on man and mouse. Consistent with the findings of Lewinsky and Stewart[20], Dependorf[10], Gach[11] and Kadnoff[20], these fibers were seen to

terminate with and without end swellings and with and without arborizations.

The periodontal membrane contained a large variety of organized nerve endings. Loops, coils and spirals similar to those described by Lewinsky and Stewart[20], Brashear[8], Bradlaw[6] and Gach[11] were noted. Knob like swellings described by Lewinsky and Stewart[19] and club shaped forms observed by Dependorf[10] were also seen.

The presence of highly organized neural structures in the interstitial spaces of the periodontal membrane (Figure 4), and encapsulated nerve endings in close association with blood vessels (Figure 3), have not been previously reported in the literature. However, the evidence presented in the photomicrographs would definitely seem to indicate their presence. These findings do not agree with the observations of Bernick[4] that no specialized nerve endings occur in the periodontal membrane.

Van der Sprenkel[28] reported that nerves in the periodontal membrane of the mouse were seen to pass through the cementum and terminate in the dentin. Bernick[2] also noted nerve fibers embedded in the cementum of the root. There was no evidence in this study to support such findings. Moreover, nerve fibers were seen to turn back from the cementum and then terminate as loops or blunt endings. This is consistent with the findings of Lewinsky and Stewart[19] on man and cat and Bradlaw[7] on man.

(b) The Gingiva

The findings of this investigation indicated that the human gingiva is extremely well innervated. These findings are in agreement with the observations of Gairns[12] and Lewinsky and Stewart[21]. Large nerve fibers in the gingiva were usually associated with blood vessels, thus agreeing with the observations of Mowry[24] on man, Gach[11] on the Syrian hamster and Bernick[2] on the monkey. There were however, numerous small bundles and single fibers that bore no such relationship.

The present study revealed that the main nerve supply of the gingiva arises from the labial or buccal and lingual or palatal nerves, and that comparatively few nerve fibers enter from the periodontal membrane. These findings are similar to the observations of Bernick[2] on the monkey and Stewart and Lewinsky[28] on man, cat, ferret, mouse, mole and the rabbit.

In the present literature, a bewildering array of terminal endings are alleged to be present in the gingiva. This investigation revealed the presence of six types of nerve termination. Two were observed in the sub-papillary connective tissue, the other four being found in the sub-epithelial papillae.

Deep in the sub-epithelial connective tissue was found a complex convoluted neural structure formed by both medullated and non-medullated nerve fibers. This organ was similar in structure to a nerve grouping already described in the interstitial spaces of the periodontal membrane. Stewart and Lewinsky[28]

are the only investigators who have reported finding a comparable structure. They suggested that since this nerve grouping is found only in the deep parts of the connective tissue which lie in close relationship to the underlying muscles, and since they have not been seen in other parts of the gingiva, that they might possibly function as muscle sense organs. Contrary to the findings of the present study, Lewinsky and Stewart were unable to locate a similar structure in the periodontal membrane.

Immediately beneath the epithelium were seen myelinated nerve fibers which break into small twigs by repeated dichotomous branchings, to end in fine arborizations. Occasionally these fibers entered the epithelium where they ramified around the cells of the deeper layers. Similar findings were reported by Gairns[12] and Lewinsky and Stewart[21].

In this investigation, Meissner corpuscles, Krause end bulbs and closed coil endings were observed in the sub-epithelial papillae. These findings are in agreement with the observations of Gairns[12] and Lewinsky and Stewart[21]. However, they are in contradiction to the findings of Held and Baud[14] on man and Bernick[5] on man and monkey. Gairns[12] suggests that the Meissner type corpuscles and Krause end bulbs are the organs of tactile sensation and cold reception respectively.

The finding of free nerve endings of various forms in the sub-epithelial papillae is consistent with the observations of

Bernick[2] on monkey and Gach[11] on the Syrian hamster. Gairns[12] suggests that free nerve endings are the organs of pain reception in the gingiva.

Gairns[12] and Lewinsky and Stewart[21] demonstrated intra-epithelial nerve endings which arise from the encapsulated endings of the sub-epithelial papillae. These endings were not seen in the present study.

Localization of Acetylcholinesterase in the Gingiva

The findings of the present investigation demonstrated that both acetylcholinesterase and non-specific cholinesterase are present in the human gingiva in almost equal amounts. However, non-specific cholinesterase was localized mainly in the deeper parts of the lamina propria, whereas acetylcholinesterase was concentrated most heavily in the connective tissue immediately beneath the epithelium and in the sub-epithelial papillae.

Krantz and Carr[18] have noted that non-specific cholinesterase is found in blood serum. Therefore, localization of this enzyme in the gingiva can probably be attributed to its presence in the blood vessels of that tissue.

However, localization of acetylcholinesterase in areas of the gingiva known to contain nerve fibers and nerve endings is strongly presumptive evidence that the cholinergic mechanism plays a role in the transmission of neural impulses in the gingiva.

In the present literature, there is a complete absence of information on this subject. For this reason it is impossible to compare the present findings with the results of others.

SUMMARY AND CONCLUSIONS

1. The innervation of the human periodontal membrane and gingiva was studied by two methods. The morphology, location and distribution of the nerves was studied using Powers method of silver impregnation. The mechanism of transmission of neural impulses was investigated using the Avery, Rapp technique for the localization of acetylcholinesterase.
2. The nerve supply of the periodontal membrane arises from branches of the dental nerves in the periapical area of the tooth and from nerve fibers entering the membrane through foramina in the alveolar bone. The fibers from the periapical area passed gingivally to form a network with the perforating branches from the alveolar bone and then continued to pass gingivally supplying all parts of the periodontal membrane.
3. At the level of the alveolar crest, anastomoses occurred between nerve fibers of the periodontal membrane of adjacent teeth and between the nerves of the periodontal membrane and subpapillary connective tissue of the gingiva. A small number of fibers from the periodontal membrane passed to the free gingiva where they terminated as free nerve endings in the papillary and reticular connective tissue.

4. A variety of nerve terminations were seen among the cells of the periodontal membrane and in close relation to the cementum and alveolar bone. These included free nerve endings with and without end swellings, complex convoluted neural structures in the interstitial spaces, specialized nerve endings in close association with blood vessels and various forms of loops and coils. No fibers from the periodontal membrane terminated in the cementum.
5. Gingival innervation is derived primarily from the labial or buccal and lingual or palatal nerves. Comparatively few nerve fibers entered the gingiva from the periodontal membrane.
6. The human gingiva is extremely well innervated and contains a large variety of nerve endings. Complex convoluted neural configurations were found deep in the lamina propria. Immediately beneath the epithelium were seen myelinated nerve fibers which ended in fine arborizations. Occasionally these fibers entered the epithelium where they ramified around the cells of the deeper layers. The sub-epithelial papillae were profusely innervated and typical Meissner corpuscles, Krause end bulbs, closed coils and free nerve endings were found in them. It is quite probable that Meissner corpuscles, Krause end bulbs, and free nerve fibers serve the same function in the gingiva as they do in the skin.

No intra-epithelial fibers were seen to arise from encapsulated nerve endings in the dermal papillae.

7. Acetylcholinesterase is found in the gingiva in areas which contain nerve fibers and nerve endings. Its presence is strongly presumptive evidence that the cholinergic mechanism plays some role in the transmission of neural impulses in the gingiva.

BIBLIOGRAPHY

1. Avery, J. K. and Rapp, R. Stain Technology. In press.

2. Bernick, S. Innervation of the primary tooth and supporting tissues of monkeys. Anat. Rec., 113:215-38, June, 1952.

3. ________. Silver Impregnation of nerves after enzymatic removal of collagenous elements. Stain. Tech., 30:253-7, Sept. 1955.

4. ________. Innervation of the teeth and periodontium of the rat. Anat. Rec., 125:185-206, June, 1956.

5. ________. Innervation of teeth and periodontium after enzymatic removal of collagenous elements. Oral Surg., Oral Med., and Oral Path. 10:323-332, March, 1957.

6. Bradlaw, R. Innervation of the teeth. Roy. Soc. Med. Sect. Odont. Proc., 29:507-18, Jan. 1936.

7. ________. The histology and histopathology of dental innervation. Roy. Soc. Med. Sect. Odont. Proc., 26:68-81, Apr. 1939.

8. Brashear, A. D. Innervation of the teeth. Am. Dent. A. J., 23:662-8, Apr. 1936.

9. Churchill, J. A., Schuknecht, H. F. and Doran, R. Acetylcholinesterase activity in the cochlea. The Laryngoscope. 66:1-15, Jan. 1956.

10. Dependorf. Innervation of the odontoblastic layer and of the dentine. Dent. Cosmos, 56:1186-7, Oct. 1915.

11. Gach, L. Nerve endings in the periodontal membrane and gingiva. Masters Thesis, University of Michigan, 1956.

12. Gairns, F. W. and Aitchison, J. Preliminary Study of Multiplicity of Nerve Endings in the Human Gums. Dental Record, 70:180-194, July - August, 1950.

13. Gairns, F. W. Sensory Nerve Endings of the Human Palate. Quart. J. of Exp. Phys. 40:40-47, Jan. 1955.

14. Held, A. J., and Baud, C. A. Innervation of the dental organ. Oral Surg., Oral., and Oral Path. 8:1262-9, Dec. 1955.

15. Koelle, G. B., and Friedenwald, J. S. A histochemical method for localizing cholinesterase activity. Proceedings of Society for Exp. Biology and Medicine. 70:617-622, Jan. - April, 1949.

16. Koelle, G. B. The elimination of enzymatic diffusion artifacts in the histochemical localization of cholinesterases and a survey of their cellular distributions. J. of Pharm. and Exp. Ther. 103:153-171, Oct. 1951.

17. ________. The histochemical identification of acetylcholinesterase in cholinergic, adrenergic and sensory neurons. J. of Pharm. and Exp. Ther. 114:167-183, June, 1955.

18. Krantz, J. C. and Carr, C. J. The pharmacologic principles of medical practice. 3rd ed., Baltimore, Williams and Wilkins, 1954. 1183 p.

19. Lewinsky, W. and Stewart, D. The innervation of the periodontal membrane. J. Anat., 71:98-103, Oct. 1936.

20. ________________. The innervation of the periodontal membrane of the cat, with some observations on the function of the end-organs found in that structure. J. Anat., 71:232-36, Jan. 1937.

21. ________________. The innervation of the human gum. J. Anat., 72:531-36, July 1938.

22. Malmgren, H. and Sylven, B. On the chemistry of the thiocholine method of Koelle. J. of Histochemistry and Cytochemistry. 3:441-445, Nov. 1955.

23. Mohiuddin, A. Fate of nerves of the deciduous teeth. J. of Anat., 84:319-323, 1950.

24. Mowry, D. P., Innervation of the gingivae, Am. Dent. A. J., 17:1050-9, June 1930.

25. Powers, M. M. The staining of nerve fibers in the teeth. J. Dent. Res., 31:383-92, June, 1952.

26. Romanes, G. J. The staining of nerve fibers in paraffin section with silver. J. of Anat. 84:104-115, 1950.

27. Stewart, D. Some aspects of the innervation of the teeth. Roy. Soc. Med. Sect. Odont. Proc., 20:55-66, Aug. 1927.

28. Stewart, D. and Lewinsky, W. A comparative study of the innervation of the gum. Roy. Soc. Med. Sect. Odont. Proc., 32:82-90, July 1939.

29. Van der Sprenkel, B. Microscopical investigation of the innervation of the tooth and its surroundings. J. Anat., 70:233-41, Jan. 1936.

APPENDIX

Figure 1. Section through the incisor root, periodontal membrane, and alveolar bone of man. Note the large fiber bundles in close association with blood vessels passing gingivally in the central portion of the periodontal membrane. B, alveolar bone; PM, periodontal membrane; and D, dentin. Romanes's stain. X200.

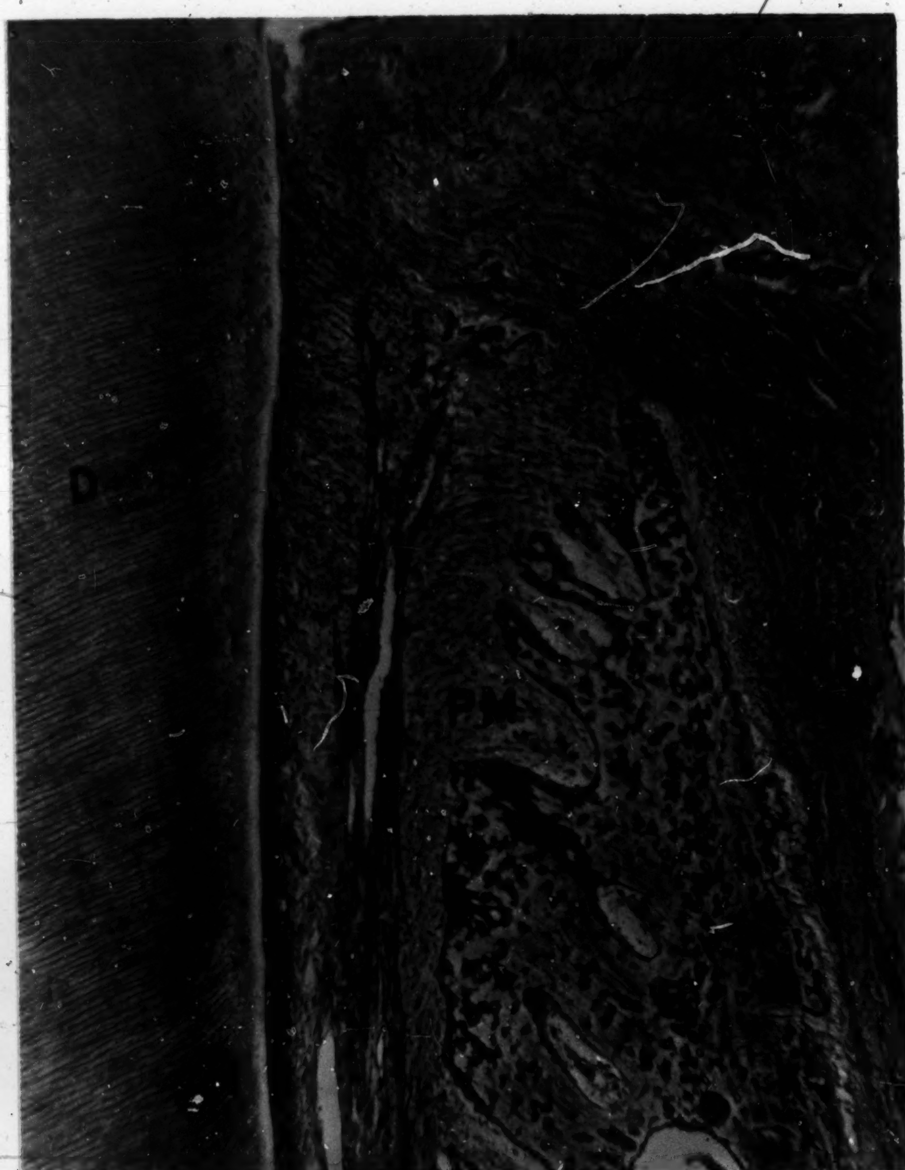

D

Figure 2. Section through the alveolar bone and periodontal membrane of man. Note the nerve fiber passing either into or out of the periodontal membrane through a foramen in the alveolar bone. N, nerve; B, alveolar bone; PM, periodontal membrane. Romane's stain. X200.

Figure 3. Section of the human periodontal membrane, cementum and dentin. Note the nerve fiber running through the periodontal membrane towards the cementum. The fiber terminates as a coil at the surface of the cementum. PM, periodontal membrane; C, cementum; N, nerve fiber; NC, nerve coil; and D, dentin. Romane's stain. X500.


B
PM
N

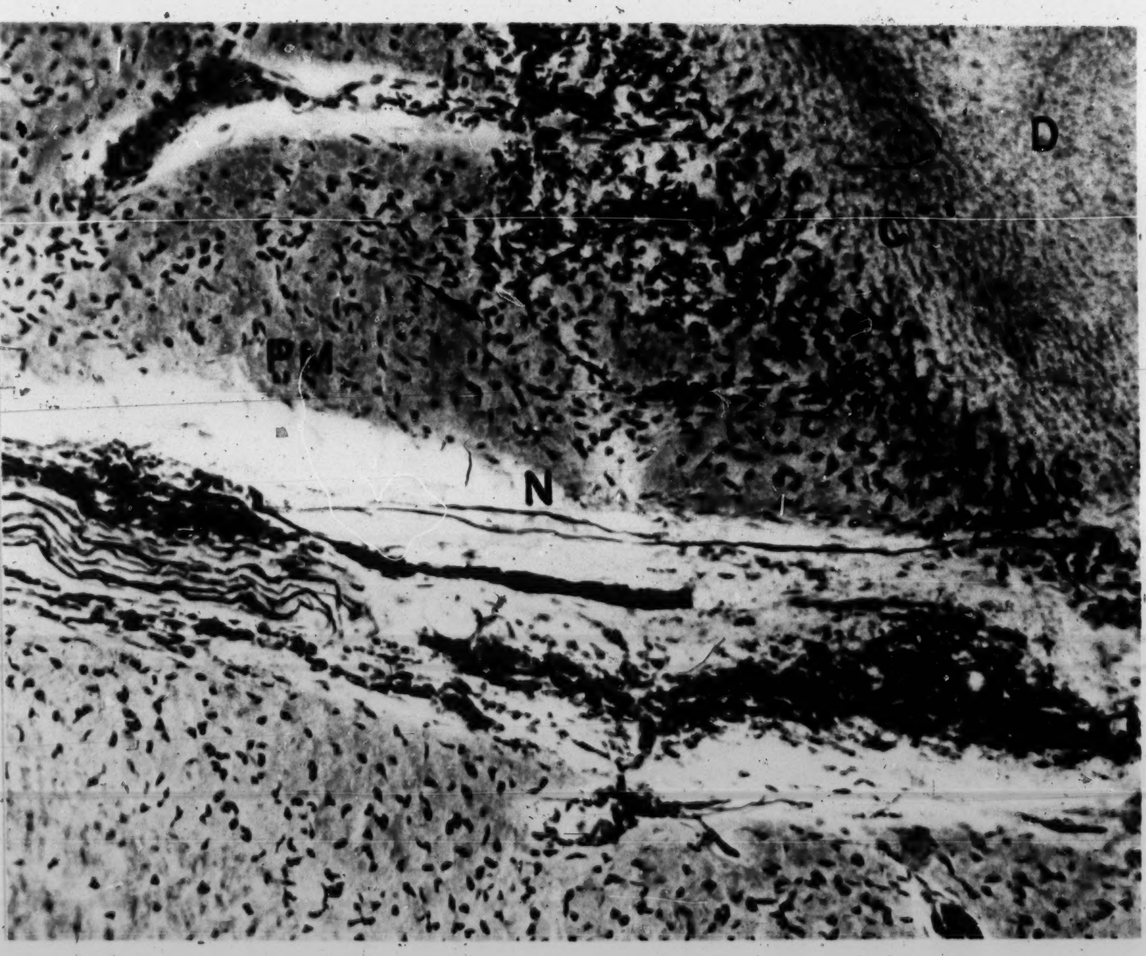
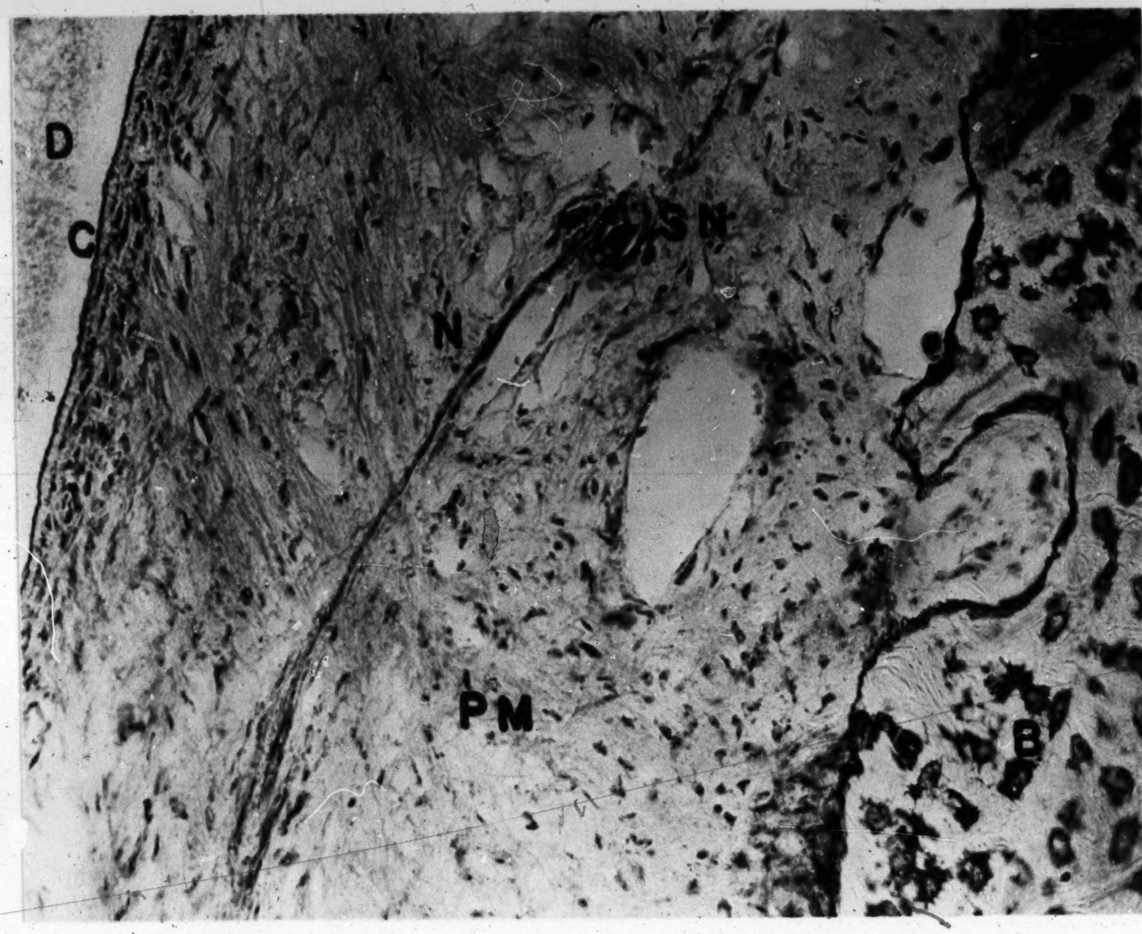

D
PM
N

Figure 4. Section through human dentin, cementum, periodontal membrane and alveolar bone. Note the nerve fiber running gingivally in close association with a blood vessel. The nerve terminates as a loop in a capsule of connective tissue. D, dentin; C, cementum; PM, periodontal membrane; N, nerve fiber; SN, specialized nerve termination; and B, alveolar bone. Romane's stain. X500.

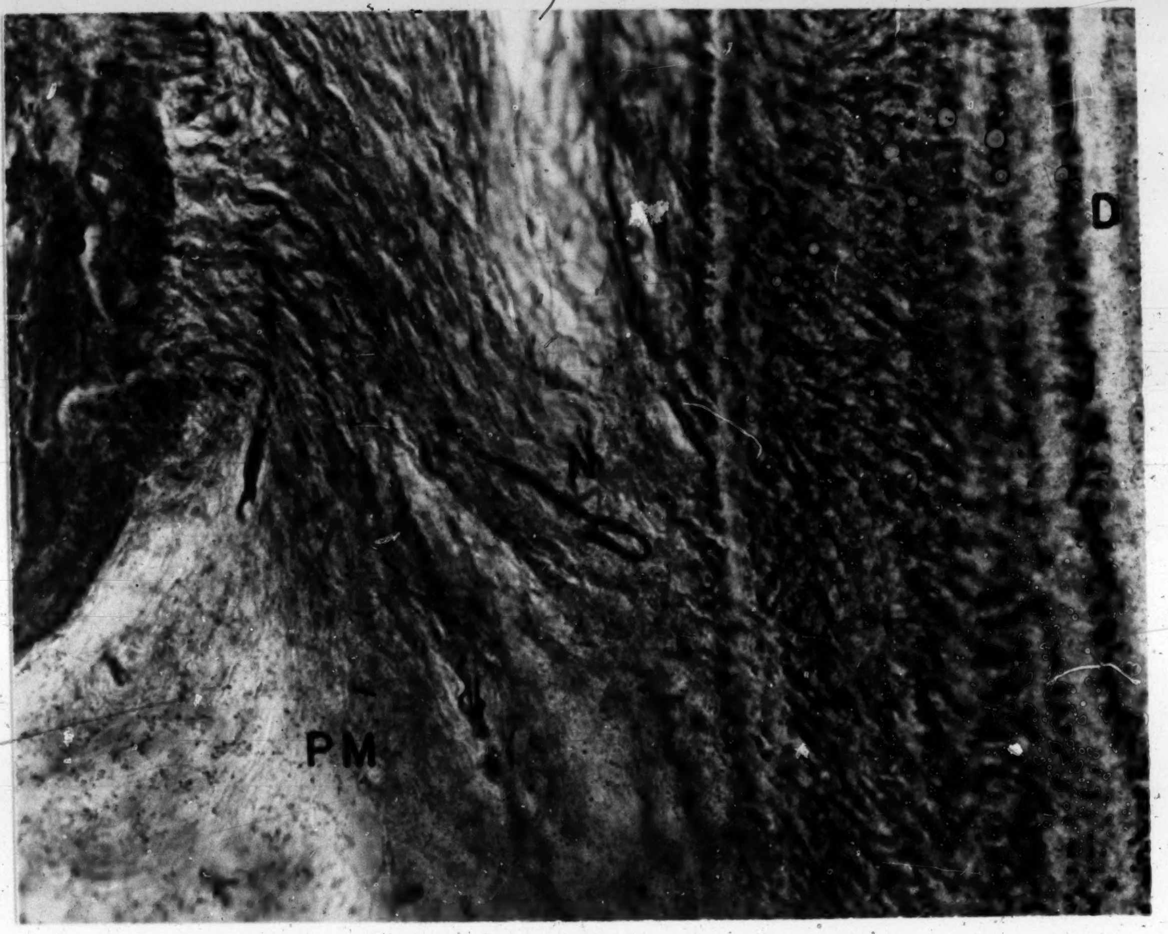
Figure 5. Section of the human periodontal membrane. Note the fine naked nerve fibrils passing either towards or away from the complex convoluted neural structure in the interstitial space. Irregular thickening of the medullated nerve fibers may be evidence of neural degeneration due to periodontal disease. P, principal fibers; I, interstitial space; MN, myelinated nerve fibers; and UN, unmyelinated nerve fibers. Romane's stain. X750.


D
C
SN
N
PM
B


UN
I
MN

Figure 6. Section through the alveolar bone, periodontal membrane, cementum and dentin of a human tooth. Note the myelinated nerve fiber approaching the cementum and turning back to terminate abruptly among the principal fibers. B, alveolar bone; N, nerve fiber; PM, periodontal membrane; C, cementum; and D, dentin. Romane's stain. X750.

Figure 7. Section through the human periodontal membrane and cementum. Myelinated nerve fiber with varicosities passing towards the cementum. Note naked nerve fibrils in association with myelinated fibers. PM, periodontal membrane; MN, myelinated nerve; UN, unmyelinated nerve fibers; and C, cementum. Romane's stain. X750.


D
N
PM


MN
C
PM
UN

Figure 8. Section through the human periodontal membrane, alveolar bone and submucosa. Note the complex neural configuration and large blood vessel in the submucosa. The nerve fibers in the periodontal membrane show evidence of degenerative changes. PM, periodontal membrane; N, nerve fibers; B, alveolar bone; NG, complex nerve grouping; S, submucosa; and BV, blood vessel. Romane's stain. X200.

Figure 9. Highly magnified view of an area of the periodontal membrane similar to the one shown in Figure 8. Note the presence of both myelinated and unmyelinated nerve fibers. PM, periodontal membrane; MN, myelinated nerve fibers; UN, unmyelinated nerve fibers; and B, alveolar bone. Romane's stain. X750.


N
PM
NG
S
BV


MN
UN
PM

Figure 10. Section through the epithelium and lamina propria of the human gingiva. Note the branching of the nerve fiber in the connective tissue immediately beneath the epithelium. E, epithelium; P, sub-epithelial papilla; and N, nerve fiber. Romane's stain. X750.

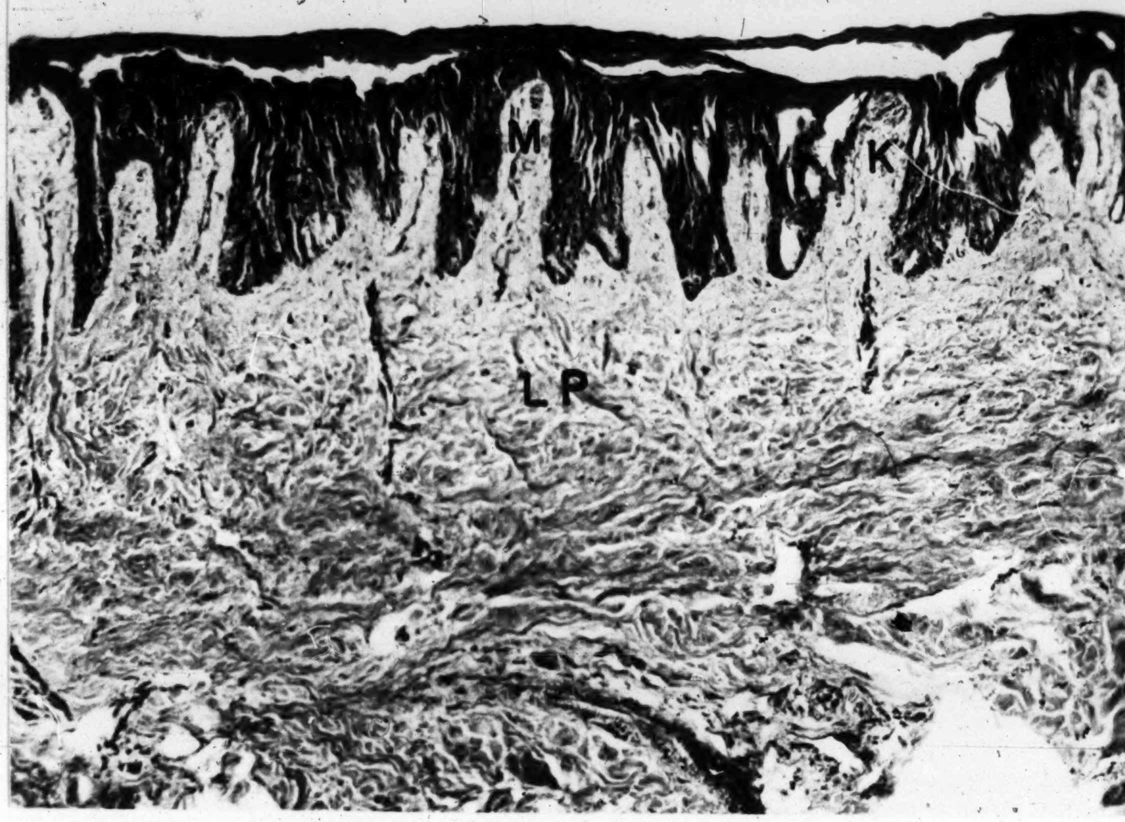
Figure 11. Section of the epithelium and papillary connective tissue of the human attached gingiva. Note either nerve fibers or a dendritic cell situated in the deeper layers of the epithelium. E, epithelium; N, nerve fibers or dendritic cell; and PC, papillary connective tissue. Romane's stain. X750.


P
N


N
PC

Figure 12. Section through the attached gingiva of man. Note the different types of specialized nerve terminations in the c.t. papillae. E, epithelium; M, Meissner type nerve ending; K, Krause end bulb; LP, lamina propria. Romane's stain. X200.

Figure 13. Highly magnified view of a Meissner type nerve ending located in the dome of a papilla. Note the central mass of irregular cells which are penetrated by the irregularly curved nerve fiber. M, Meissner type nerve ending; PC, papillary connective tissue; N, nerve fiber; and E, epithelium. Romane's stain. X1800.


M
K
LP

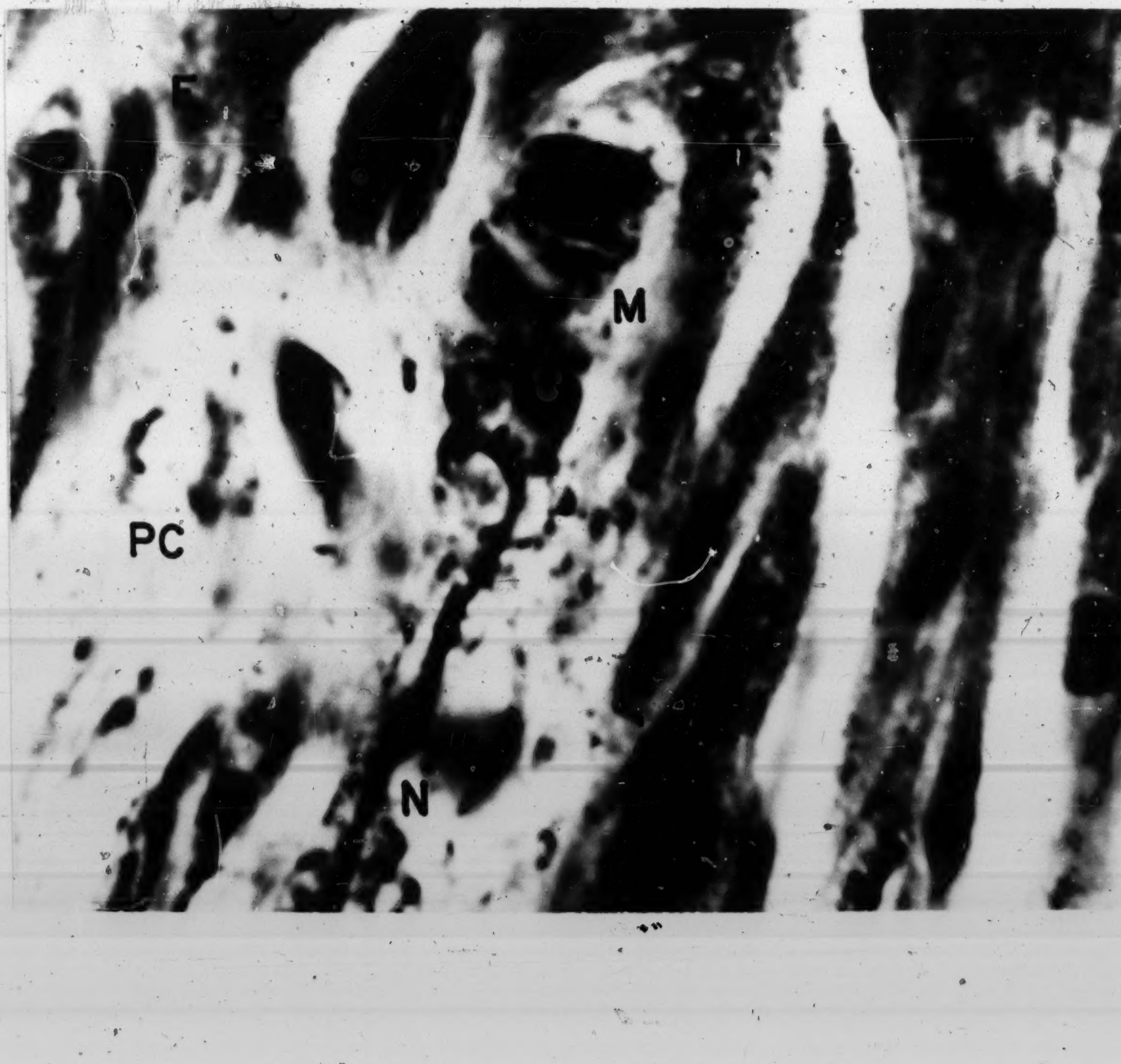

F
M
PC
N

Figure 14. Section through three sub-epithelial papillae of the human gingiva. Note the nerve fiber passing towards the dome of the sub-epithelial papilla, where it terminates as a closely wound coil. N, nerve fiber; C, closely coiled nerve ending; and PC, papillary connective tissue. Romane's stain. X500.


C
N
PC

Figure 15. Section through the epithelium and sub-epithelial papillae of the human gingiva. Note the nerve fiber in the sub-epithelial papilla which terminates as a closely coiled, knob like ending. C, closely coiled nerve ending; and PC, papillary connective tissue. Romane's stain. X500.

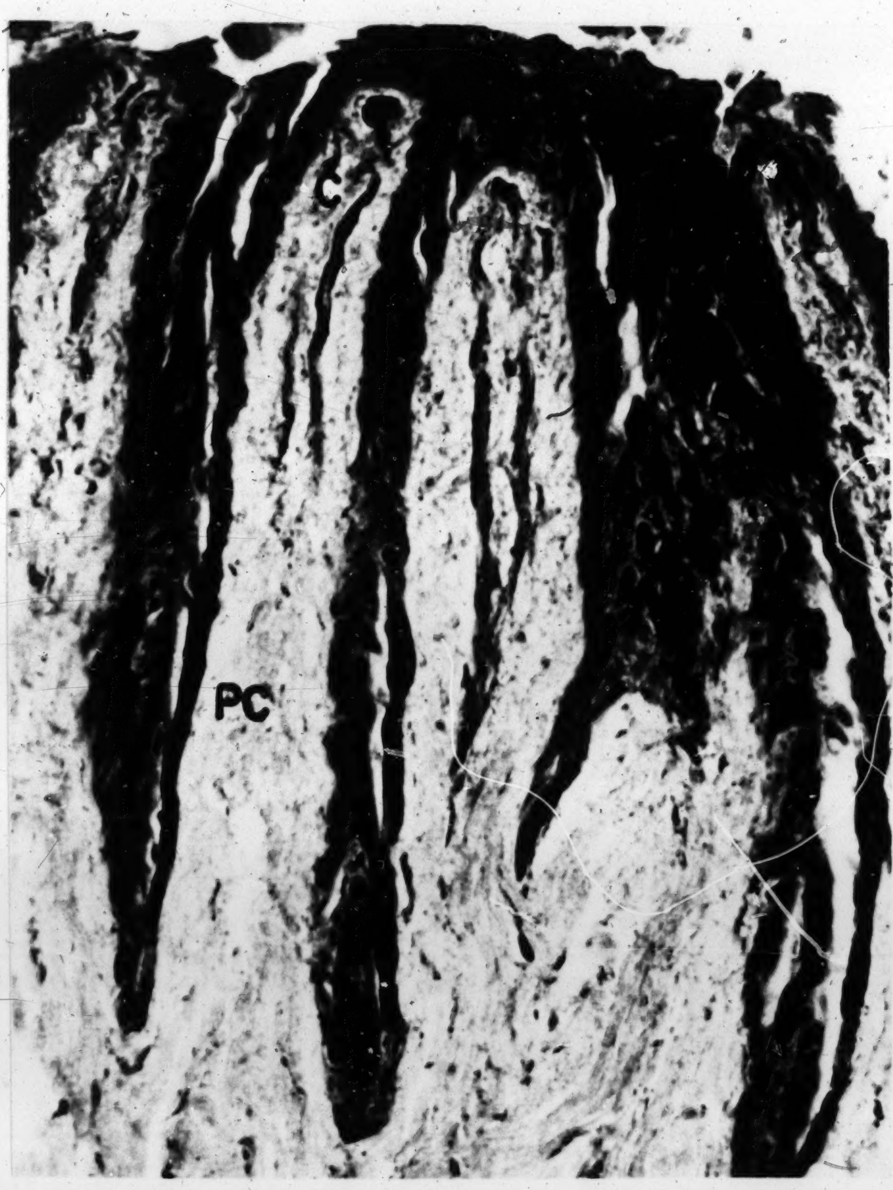
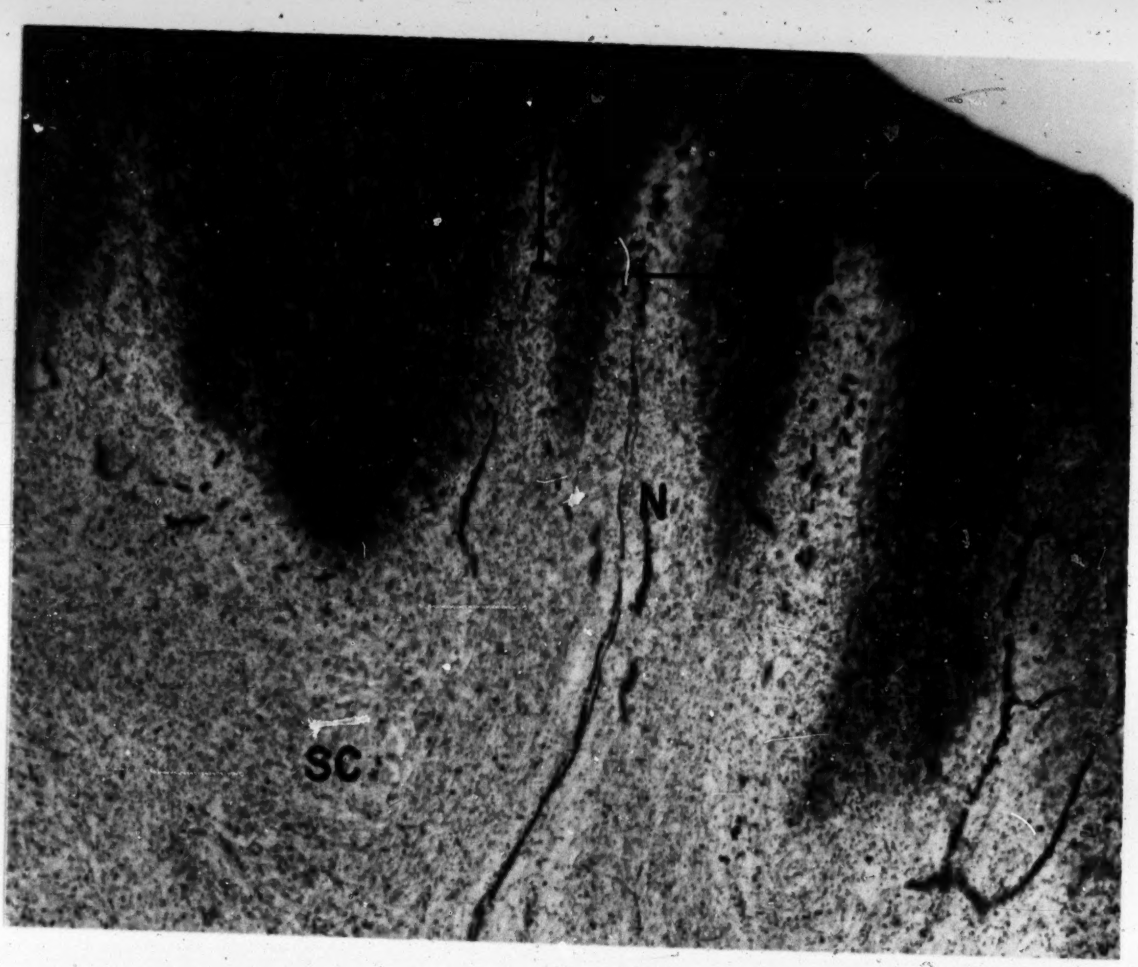

c
PC

Figure 16. Section through a sub-epithelial papilla of the human attached gingiva. Another example of the frequently seen Meissner type nerve ending. It is assumed that these endings fulfil the same function in the gingiva as in other sites of the skin, namely tactile sensation and localization. MC, Meissner corpuscle; and P, sub-epithelial papilla. Romane's stain. X750.


MC
P

Figure 17. Section through the epithelium and sub-epithelial connective tissue of the human free gingiva. Note the nerve fiber from deep in the lamina propria passing into the sub-epithelial papilla where it terminates. Outlined area shown in Figure 18. N, nerve fiber; SC, sub-epithelial connective tissue; and E, epithelium. Romane's stain. X200.

Figure 18. Highly magnified view of the area outlined in Figure 17. Note the blood cells and open mesh-work of nerve fibers in the dome of the sub-epithelial papilla. P, sub-epithelial papilla; BC, blood cells; and N, nerve ending. Romane's stain. X750.


N
SC

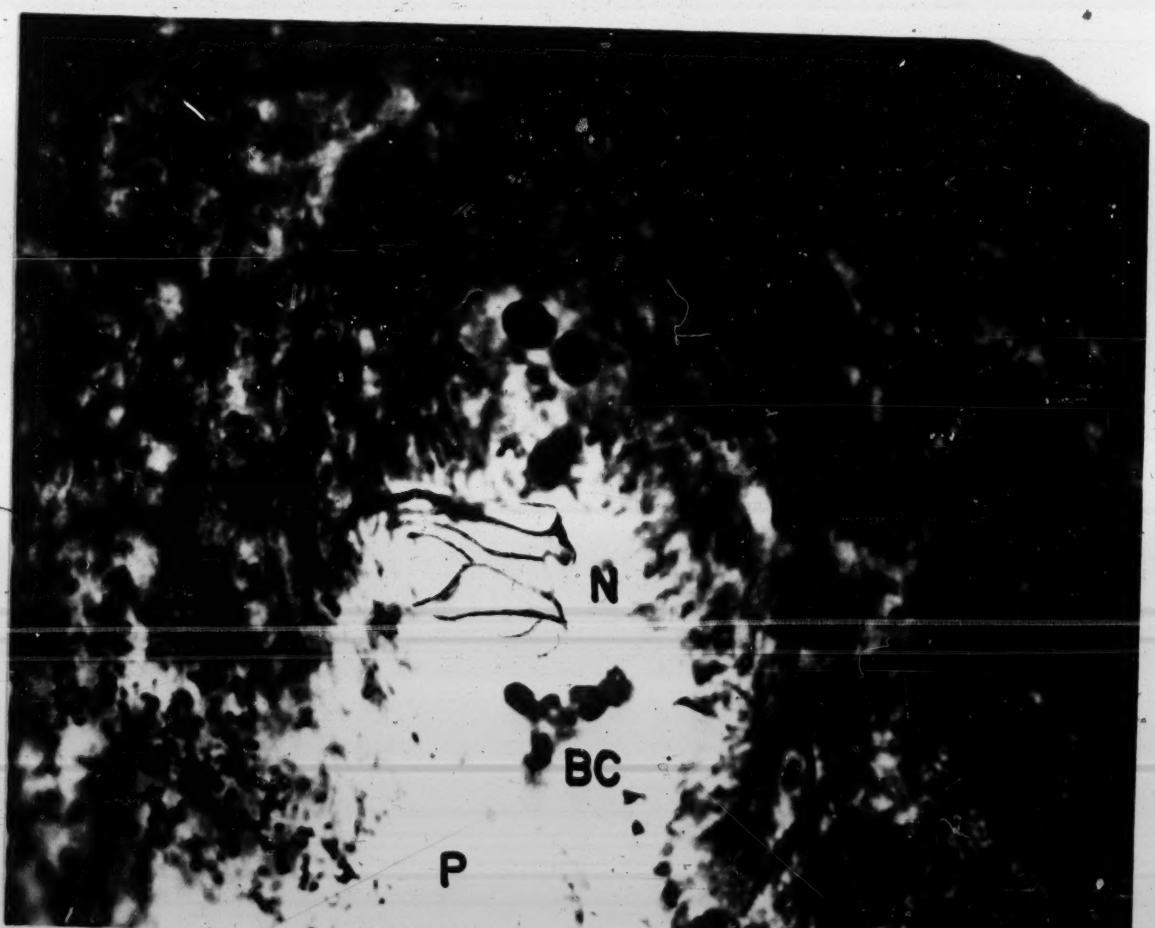

N
BC
P

Figure 19. Section of the human gingiva incubated without a substrate to act as a control for the histochemical localization of acetylcholinesterase. Note the absence of any darkly stained areas. E, epithelium; and LP, lamina propria. Avery, Rapp technique. X200.

Figure 20. Section of the human gingiva incubated with acetylthiocholine with no enzyme inhibition. Note the numerous, diffuse, darkly stained areas indicating deposits of copper sulfide. These areas which indicate the presence of both specific and non-specific cholinesterase are concentrated most heavily in the lamina propria. E, epithelium; L, lamina propria and C, copper sulfide precipitate. Avery, Rapp technique. X200.


E
A
LP


E
L
C

Figure 21. Section of the human gingiva treated with di-isopropyl fluorophosphate inhibitor to inactivate the non-specific cholinesterase and then incubated with acetylthiocholine substrate to demonstrate acetylcholinesterase. Note that the stained areas are more localized than those shown in Figure 20 and are concentrated most heavily in the sub-epithelial papillae. E, epithelium; L, lamina propria; and C, copper sulfide deposits. Avery, Rapp technique. X200.

E
C
L